also present as a nitrogen source in the MS medium. Therefore, in this case, the concentration of nitrate ion decreased to 53% of the original MS medium. By the reduction in ammonium nitrate to $^1/_{10}$ of MS medium, the ratio of ammonium ion to nitrate ion becomes very low based on mol concentration. Similar results have been obtained by Sriskandarajah et al. (1990). From these results, it is concluded that such a marked reduction in the concentration of ammonium ion compared with that of nitrate ion is a main factor on the enhancement of in vitro rooting of the plants in these experiments.

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Plant Tissue Culture and Plant Improvement: Some Recent Findings from Experiments with *Schlumbergera*, *Hatiora* (*Rhipsalidopsis*), and *Campanula*©

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INTRODUCTION

Plant tissue culture techniques have applications to a wide range of species and such usage has increased dramatically over the past twenty or so years. Techniques used include regeneration of cells, tissues, protoplasts, organs, embryos, ovules, microspores, anthers, etc. More recently, applications of these techniques have been extended to improve existing cultivars. Tissue culture techniques applied to plant improvement gives the opportunity to introduce genes across species. In addition, selection of somaclones through variation created by the tissue culture techniques themselves or those induced by mutagenic agents could also be used for creating new varieties.

Several ornamental plants suffer from sensitivity to exogenous ethylene, and it results in premature bud drop and early flower senescence. Anti-ethylene compounds such as silver thiosulfate, which are harmful and costly, are being used by the growers to reduce the above problems. However, the growers are very keen to reduce or

stop using such chemicals. One way to over come ethylene sensitivity is to introduce the mutant *etr1-1* gene, which confers insensitivity to ethylene into the plants.

To achieve the above aims using tissue culture techniques, it is essential to establish efficient regeneration and transformation systems for the plants concerned. This paper reports some recent findings from the work carried out on regeneration and transformation of some selected cultivars of *Schlumbergera, Hatiora*, and *Campanula carpatica.*

INDUCTION OF VARIATION IN TWO CACTI SPECIES

Potted plants of these two cacti species (*Schlumbergera* and *Hatiora*) are economically important in Denmark and many other countries. New cultivars are always sought and production of ethylene insensitive plants will be of great value. Cacti have a slow growth rate, low rates of seed production and germination, and, therefore, production of new genotypes through conventional breeding methods can be cumbersome. Tissue culture techniques were tried out to establish a regeneration system in order to use this method for mutation and genetic transformation.

Initial establishment of all cactus cultures in vitro was successful as the phylloclade explants have a smooth surface and 1.5% active chlorine was sufficient to eliminate contaminants. All methods for the production of in vitro cultures are described in a publication by Sriskandarajah and Serek (2004). Although it was easy to establish initial cultures, it was extremely difficult to establish a regeneration system through adventitious shoot formation. The phylloclade explants produced axillary shoots with ease with suitable hormonal treatment, but they were difficult-to-regenerate through adventitious shoots. However, after some in vitro manipulations, it was possible to induce adventitious shoots in long-term phylloclade and callus cultures. There was a significant improvement in adventitious shoot formation by increasing numbers of subcultures of the axillary shoots produced in vitro. The axillary shoots generated from explants were either subcultured to produce successive generations of axillary shoot cultures or made into phylloclade explants and treated for adventitious shoot formation. Only a few adventitious shoots formed in the early stages of cultures, but after the third subculture, the rate of adventitious shoot formation was more than 80% in *Hatiora* (cultivars CB4 and CB5) cultures. There were as many as 12 shoots from each explant. Similarly, callus cultures also became regenerative with increasing period in culture. However, all cultures from *Schlumbergera* (cultivar Thor-Olga and Russian Dancer) were much less regenerative when compared to those from *Hatiora*. The detailed results and related discussion of this work were reported in a recent publication (Sriskandarajah and Serek, 2004).

Using the above method, adventitious shoots were produced from gamma-ray-treated phylloclade explants and callus cultures. Gamma- or X-ray-treated seeds failed to grow. All shoots generated afterwards were induced to root and sent to the growers for further culture in soil. Any resulting variation will be verified when the plants start flowering. The above methods are currently being used to transform cultures with *etr1-1* gene.

PRODUCTION OF CAMPANULA PLANTS WITH ETHYLENE RESISTANCE

Campanula species are popular as potted plants. The flowers of these plants are very sensitive to ethylene. In addition, the withered flowers instead of dropping

off tend to remain on the plants giving them an undesirable appearance. Commercial growers spray such plants with anti-ethylene compounds to overcome this problem. As stated above, the main approach of the present study was to introduce the *etr1-1* gene in some important cultivars of *C. carpatica* in order to obtain ethylene-insensitive plants.

Efficient regeneration protocols were established using hypocotyls and cotyledon explants (Sriskandarajah et al., 2001). Later on, efficient transformation methods were developed using an *Agrobacterium* strain containing the *etr1-1* gene under the control of the flower specific promoter *fbp1*. We found that several factors could affect the transformation efficiency. The age of the seedling from which the explants were taken for transformation work and the growth conditions provided for the seedling growth had a significant influence on transformation efficiency. Hypocotyls taken from 12-day-old seedlings grown in the dark were the most productive, and up to 25% of hypocotyls produced transformed shoots. Older seedlings produced mainly transformed callus. Factors such as medium for co-cultivation and incubation also had significant influence on transformation. Early stages of the transformed shoots were very slow to extend. Manipulations with media constituents helped in speeding up the growth of shoots. Sriskandarajah et al. (2004) have reported the respective results and discussion in a publication.

The above-mentioned shoots were then induced to root and transferred to soil in the greenhouse. They were grown up to maturity and tested for ethylene sensitivity. Several of these transgenic plants expressed high level of ethylene tolerance. The plants are presently being analyzed for confirmation by further molecular tests, and all these results will soon be included in a forthcoming publication.

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